5-27-94

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 128897. Lambda Cyhalothrin (Karate®)/Cyhalothrin. Request to Raise NOEL for Chronic Dog Study and Response to RfD Committee Questions on Mouse Oncogenicity Study

PC Code 128897
Tox. Chem. No. 725C, 271F
MRID Nos. 400279-02, 00154795, 458901-01

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Background and Request:

The Health Effects Division RfD/Peer Review Committee met on February 12, 1993 to reassess the Reference Dose for Cyhalothrin / Lambdacyhalothrin (Karate®) in light of additional information provided by the Toxicology Branch I (TB-I) on the reproduction study conducted with cyhalothrin. At that meeting, the NOEL for the reproduction study was raised from less than 0.5 mg/kg/day to 1.5 mg/kg/day and the NOEL for the 1-year dog study conducted with lambdacyhalothrin was lowered from 0.5 mg/kg/day to 0.1 mg/kg/day, based on signs of neurotoxicity observed at 0.5 mg/kg/day. The RfD was also revised accordingly and is now based on the NOEL from the dog study using an uncertainty factor of 100. The RfD was calculated to be 0.001 mg/kg/day. In addition to these changes, the Committee questioned whether or not the mouse oncogenicity study conducted with cyhalothrin was tested at sufficiently high dose levels and was concerned about the rate of mammary tumors. They requested any relevant data supporting the dose levels selected for the study and any available historical

control data for the mammary tumors that were observed in the study.

The Registrant has submitted several addenda to the 1-year dog study conducted with lambda cyhalothrin, one of which corrects two transcription errors that were made in the ataxia summary table. This table now accurately summarizes the original individual animal data tables (the corrected page from the ataxia summary table is reproduced later in this memorandum). In addition, the Registrant has submitted comments on the incidences of fluid feces in dogs tested with pyrethroid chemicals (see Appendix II) and on the convulsions in 2 dogs at 0.5 mg/kg/day (see Appendix III). **In light of these corrections and additions, the Registrant has requested that the HED RfD/Peer Review Committee reconsider the NOEL and LEL for the dog study, including in that decision the results from the 6-month dog study conducted with cyhalothrin, thus effectively raising the RfD value from 0.001 mg/kg/day to 0.005 mg/kg/day.** As a point of reference, cyhalothrin consists of a mixture of 4 stereoisomers and lambdacyhalothrin is a mixture of 2 of the 4 cyhalothrin stereoisomers (see discussion on Agency position on these two stereoisomer mixtures from memorandum dated 8/25/93 from G. Ghali to G. LaRocca).

The Registrant has also submitted a 28-day range-finding study (see Appendix V for Data Evaluation Record), a rationale for the selection of the dose levels for the mouse oncogenicity study (see Appendix IV), and some comments concerning the incidence of mammary adenocarcinomas in the study (see later text). The historical control data were originally submitted with the study itself. **With these additional data, it is hoped that the Committee will be able to complete its assessment of the carcinogenicity studies on cyhalothrin.**

Summary Review:

One-Year Dog Study Conducted with Lambdacyhalothrin

In this study, six dogs/sex/dose level received lambda cyhalothrin (96.5%) via oral administration in gelatin capsules. The test chemical had been dissolved in corn oil prior to placement in the capsules. The following dose levels were tested: 0, 0.1, 0.5 or 3.5 mg/kg/day. The following parameters were measured and/or recorded: daily clinical observations, body weights, food consumption, ophthalmological examinations, clinical biochemistry, urinalysis, gross necropsy and microscopic examinations.

At 0.1 mg/kg/day 1 male had slight ataxia (unsteady gait, weeks 5-8) on one occasion and 1 female had blood stains on the pen floor with no obvious cause in week 27. The Registrant states that "'slight ataxia' was the term used in the reporting

laboratory (CTL) at the time to describe the observation of 'unsteady gait' which is not a frank and unequivocal sign of ataxic muscular incoordination." Fluid feces were observed, but the incidences were either similar to or less than the incidences in the control group (see table below).

At 0.5 mg/kg/day, there were 2 dogs with gait abnormalities. In the first dog (male # 28), the first time was in week 2, 7 hours post dosing. The effect was listed in the report as stiffened hind limb movement and flicking of the tarsus was apparent when trotting. It was not listed as ataxia. The second time occurred 2 days later when similar limb movements were observed again immediately after dosing but were reported as ataxia. The second dog was female dog # 34. Slight ataxia (unsteady gait) was observed 4 times during week 9. It was recorded, but there was no description of the ataxia in individual animal data. In referring to these two cases, the Registrant states that "isolated incidences of "slight ataxia" were recorded in 2 of the 12 dogs (1 male and 1 female), or on 5 of 13,104 observations made in the 0.5 mg/kg/day dose group (i.e. in less than 0.04% of the observations)."

Convulsions were observed in 2 other dogs (male # 26 in weeks 52 (30 seconds while being carried) and 53 (30 seconds while being carried) and male # 27 in week 51 (2-3 minutes after being placed in metabolism cage; for 30 seconds 5 minutes later; 3 minutes next morning when taken out of metabolism cage). **These were originally listed as severe ataxia in the summary table, which is an error.**

Blood stains on the pen floor with no obvious cause were seen in 2 dogs.

The frequency of fluid feces was increased in 1 dog (female # 33). The incidences of fluid feces observations for this dose group were somewhat increased over controls (see table below).

At 3.5 mg/kg/day, 1 male was killed <u>in extremis</u> in week 46 because of severe ataxia and convulsions which persisted over a period of 2 days, even though the dosage was withheld during this period. Ataxia was observed in all dogs, and was apparent from the first week in some dogs. The ataxia was observed 3-7 hours post-dosing. Other clinical signs included muscle tremors, convulsions (3 males: weeks 46, 25 and 37 and 1 female: # 43 in text but could not find reference to convulsions in individual animal data), occasional subdued behavior, worn or bleeding claws, regurgitation of food during the first 2 weeks, fluid feces in all dogs and occasional decrease in food consumption.

The toxicological significance of the fluid feces in unknown. It was not stated in the report the number of hours (days) after dosing the liquid feces were observed. The

Registrant's report states the following: "True diarrhea was not a feature of the condition and the dogs often passed normal feces on the days when fluid feces were also observed. The passing of fluid feces did not reflect the general health of the animals and was not associated with histopathological lesions in the alimentary tract. Increased incidences of fluid feces have been reported in studies where, for example, cypermethrin has been administered to dogs. In this case the compound was administered in capsules but where it has been included in the diet there was no evidence of fluid feces, even at concentrations which were highly toxic. It is reasonable to conclude from the above examples that fluid feces are produced as a result of the method of administration. When given in capsule a bolus of the test compound is presented to the gastrointestinal tract and this may produce a direct local (irritant) effect which stimulates the production of liquid feces." For additional comments on fluid feces provided by the Registrant, refer to Appendix II.

TB-I notes that data are available on another pyrethroid where this is the case as well: fluid feces were observed when the chemical was administered in corn oil via capsule and were not observed when administered in the diet.

The Registrant submitted comments on the convulsions in the 2 male dogs at the 0.5 mg/kg/day dose level. In their response, they stated that convulsions have been observed in 2 control dogs from 2 other studies, a male and a female, 1 each from each of the studies. In these studies, 1 male dog convulsed during bone marrow sampling and 1 female dog convulsed on being taken into the clinical examination room, on week 53 of the study, just prior to termination. The situation with this dog was similar to the one with the lambda-cyhalothrin dog study. The Registrant also stated that convulsions were observed with other dogs being tested with chemicals that were known not to be neurotoxic (see Appendix III for details).

The following tables are to be appended to the original Data Evaluation Record (DER) in order to provide a more complete assessment. The first table is the last page of the ataxia summary table (corrected version).

PP321: 1 YEAR ORAL DOSING STUDY IN DOGS

Table 3 - continued

INCIDENCE AND SEVERITY OF ATAXIA (INDIVIDUAL ANIMALS)[1]

Treatment (mg PP321/kg/day)	Sex	Animal No.	Duration Weeks 49-52(53)		
			SLIGHT	MODERATE	SEVERE
0.1	Male	14	0	0	0
0.5	Male	26	0	0	0
		27	0	0	0
		28	0	0	0
	Female	34	0	0	0
3.5	Male	37	3	1	0
		38	0	0	0
		39*	-	-	-
		40	9	1	6
		41	0	0	0
		42	7	0	3
	Female	43	2	0	2
		44	0	0	0
		45	0	0	0
		46	0	0	0
		47	0	0	0
		48	0	0	0

[1]Expressed as number of observations/4 weeks.

Slight = unsteady gait
Moderate = Incoordinated gait
Severe = Straddled gait/recumbency

There was no incidence of ataxia in control animals

* Killed in week 46.

5

Intergroup Comparison of the Incidences of Liquid Feces Over Entire Study

Incidences	Dose Levels (mg/kg/day)			
	0	0.1	0.5	3.5
		Males		
# Observations/	45[a]	31[b]	98[c]	1373
# Dogs Affected	5	6	6	6
		Females		
# Observations/	32	34[d]	216[e]	1234
# Dogs Affected	6	6	6	6

[a]27 incidences in one animal.
[b]18 incidences in one animal.
[c]39 incidences in one animal.
[d]25 incidences in one animal.
[e]161 incidences in one animal.

Intergroup Comparison of Bodyweight Gain (Kg) From Start of Study - Males

Weeks	Treatment (mg/kg/day)			
	0	0.1	0.5	3.5
Initial Wt.	11.98	11.97	12.05	12.00
4	0.47	0.55	0.70	0.57
8	1.05	1.00	1.28	0.97
12	1.37	1.38	1.63	1.42
16	1.67	1.70	2.05	1.67
20	1.93	1.90	2.28	2.03
24	1.92	2.02	2.38	2.17
28	1.90	1.92	2.43	2.33
32	1.97	2.02	2.65	2.57
36	1.97	1.93	2.67	2.67
40	2.10	2.00	2.62	2.85
44	2.08	1.87	2.70	2.93
48	2.05	1.82	2.55	2.63
52	1.88	1.68	2.42	2.48
Final Weight	13.87	13.65	14.47	14.51

Intergroup Comparison of Bodyweight Gain (Kg) From Start of Study - Females

Weeks	Treatment (mg/kg/day)			
	0	0.1	0.5	3.5
Initial Wt.	10.72	10.80	10.58	10.72
4	0.68	0.80	0.88	0.77
8	0.92	1.33*	1.27	1.27
12	1.20	1.65*	1.53	1.52
16	1.50	2.23*	1.98	1.70
20	1.68	2.48*	2.20	1.87
24	1.83	2.53	2.32	1.67
28	1.90	2.72	2.42	1.72
32	2.03	2.72	2.52	1.92
36	2.03	2.92	2.65	2.00
40	2.17	3.02	2.85	2.25
44	2.08	2.98	2.78	2.12
48	2.08	2.95	2.70	2.03
52	2.17	2.88	2.82	1.77
Final Weight	12.88	13.68	13.40	12.48

*Statistically significantly different from control ($p < 0.05$).

Intergroup Comparison of Plasma Triglycerides (mg/100ml)

Weeks	Treatment (mg/kg/day) 0	0.1	0.5	3.5
		Males		
Pre-experimental	32.3	31.3	31.8	31.0
4	38.9	37.9	37.9	47.7
13	28.8	34.4	29.4	35.1
26	30.9	34.2	33.5	37.0
39	31.5	29.0	29.0	39.7
52	27.6	32.5	27.5	37.3
		Females		
Pre-experimental	36.7	35.3	35.2	31.7
4	35.0	34.6	33.5	43.2
13	34.3	26.4	24.6*	31.1
26	38.6	29.5	33.4	39.6
39	35.8	34.6	30.1	40.0
52	36.1	39.5	30.9	43.8

*Statistically significantly different from controls ($p < 0.05$)

Intergroup Comparison of Plasma Cholesterol (mg/100 ml)

Weeks	Treatment (mg/kg/day) 0	0.1	0.5	3.5
		Males		
Pre-experimental	155	166	155	179
4	150	151	139	149
13	147	147	133	133
26	152	157	140	130
39	134	143	132	122
52	129	132	129	104
		Females		
Pre-experimental	165	162	156	151
4	139	127	139	137
13	135	136	138	134
26	149	151	158	138
39	124	138	124	141
52	139	142	129	132

*Statistically significantly different from controls ($p < 0.05$)

Intergroup Comparison of Plasma Sodium (mEq/l)

Weeks	Treatment (mg/kg/day) 0	0.1	0.5	3.5
		Males		
Pre-experimental	153	153	152	153
4	147	147	147	145
13	148	149	148	147
26	157	157	156	154*
39	153	153	152	152
52	158	158	156*	155**
		Females		
Pre-experimental	153	154	153	154
4	149	148	148	146*
13	150	149	150	148*
26	160	159	158*	157**
39	153	153	154	152
52	157	158	159*	157

*Statistically significantly different from controls ($p < 0.05$)
**Statistically significantly different from controls ($p < 0.01$)

Intergroup Comparison of Plasma Potassium (mEq/l)

Weeks	Treatment (mg/kg/day) 0	0.1	0.5	3.5
		Males		
Pre-experimental	4.63	4.67	4.85	4.30
4	4.83	4.77	4.82	4.69
13	4.52	4.55	4.62	4.58
26	4.80	5.07	4.84	4.74
39	4.59	4.58	4.37	4.25*
52	4.55	4.49	4.40	4.25*
		Females		
Pre-experimental	4.58	4.60	4.63	4.58
4	4.42	4.62	4.50	4.54
13	4.55	4.60	4.60	4.85
26	4.87	5.17	4.78	4.98
39	4.34	4.48	4.49	4.16
52	4.48	4.62	4.45	4.50

*Statistically significantly different from controls ($p < 0.05$)

Intergroup Comparison of Plasma Calcium (mg/100 ml)

Weeks	Treatment (mg/kg/day) 0	0.1	0.5	3.5
		Males		
Pre-experimental	11.5	11.7	11.3	11.5
4	11.1	11.2	11.1	11.1
13	10.6	10.8*	10.7	10.6
26	10.7	10.8	10.7	10.6
39	10.5	10.4	10.5	10.5
52	10.4	10.4	10.4	10.2
		Females		
Pre-experimental	11.2	11.5	11.3	11.5
4	11.2	11.1	11.3	10.9
13	10.9	10.9	10.9	10.8
26	10.9	11.0	11.0	10.7
39	10.5	10.6	10.6	10.5
52	10.5	10.5	10.5	10.2

*Statistically significantly different from controls ($p < 0.05$)

Intergroup Comparison of Urine Biochemistry

Urine pH	Dose Level (mg/kg/day) 0	0.1	0.5	3.5
Males				
Pre-Experimental	6.93	6.46	6.85	6.76
Week 25	6.94	6.83	6.88	7.04
Week 51	7.67	7.18	7.38	7.25
Females				
Pre-Experimental	6.47	6.50	6.67	6.49
Week 25	6.77	6.86	6.58	6.87
Week 51	7.37	7.38	7.31	7.14
Urine Specific Gravity				
Males				
Pre-Experimental	1.032	1.029	1.028	1.033
Week 25	1.039	1.039	1.042	1.034
Week 51	1.040	1.039	1.043	1.041
Females				
Pre-Experimental	1.032	1.026	1.029	1.033
Week 25	1.041	1.040	1.035	1.034
Week 51	1.038	1.039	1.040	1.044

Intergroup Comparison of Absolute Organ Weights (g)

Organ	Treatment (mg/kg/day) 0	0.1	0.5	3.5
	Males			
Kidney	59.1	58.9	60.3	67.9*
Liver	389	401	408	445
Testes	30.2	30.2	29.1	28.0
Adjusted for bodyweight	30.8	31.4	28.1	26.8
	Females			
Liver	357	373	380	406

*Statistically significantly different from controls ($p < 0.05$).

Intergroup Comparison of Microscopic Findings - Males

Observation	Treatment (mg/kg/day) 0	0.1	0.5	3.5
Kidney				
# Examined	6	6	6	6
Unilateral pyelitis	0	0	1	1
Liver				
Congeries of pigment laden Kupffer cells	0	0	4	1
Testes				
Atrophy of seminiferous epithelium	0	0	1	0
Orchitis - unilateral	0	0	0	1

Intergroup Comparison of Microscopic Findings Females

Observation	Treatment (mg/kg/day)			
	0	0.1	0.5	3.5
Kidney				
# Examined				
Unilateral pyelitis	0	0	2	1
Bilateral pyelitis	0	2	0	1
Liver				
Congeries of pigment laden Kupffer cells	4	3	3	3
Mammary Gland				
Hemorrhage	0	0	1	0

6-Month Dog Study Conducted With Cyhalothrin

In this study, six dogs/sex/dose level received cyhalothrin (technical) via oral administration in gelatin capsules. The test chemical had been dissolved in corn oil prior to placement in the capsules. The following dose levels were tested: 0, 1.0, 2.5 or 10.0 mg/kg/day. The following parameters were measured and/or recorded: daily clinical observations, body weights, food consumption, ophthalmological examinations, neurological examinations, clinical biochemistry, urinalysis, gross necropsy and microscopic examinations.

At 1.0 mg/kg/day, liquid feces in both sexes combined were observed at an increased rate over the control group (see table below). This was the only effect observed at this dose level. In the original DER it was not considered to be of toxicological significance.

At 2.5 mg/kg/day, liquid feces were observed at an increased rate over the control group (both sexes combined). No other effects were observed. At this dose level, the liquid feces was considered to be a toxicological effect in the original DER.

At 10.0 mg/kg/day, liquid feces were observed at an increased rateover the control group (both sexes). In addition, the following effects were observed: increase in water consumption during first 4 weeks, vomiting, usually within a few hours following dosing and occasional unsteadiness and/or muscular trembling. During week 2, head shaking and excessive salivation were observed in several dogs. These signs were observed only occasionally during the subsequent test weeks. One male had more severe signs. During the second week, excessive salivation and head shaking were noted. On day 14, 3 hours post-dosing, the dog was in a state of collapse, stiff limbed and frothing at the mouth with the presence of vomitus. It recovered in 6 hours. In the following weeks with this dog, there were periods of head shaking, salivation, loss of appetite, episodes of collapse, muscular spasms, marked incoordination, vocalization and one episode of convulsive behavior (week 8).

The following tables are to be appended to the original Data Evaluation Record (DER) in order to provide a more complete assessment.

Intergroup Comparison of the Incidences of Liquid Feces (Both Sexes Combined)

Incidences	Dose Levels (mg/kg/day)			
	0	1.0	2.5	10.0
# Observations/ # Dogs Affected	31/ 2♂; 6♀	389/ 6♂; 6♀	671/ 6♂; 6♀	1008/ 6♂; 6 ♀

Initial Bodyweights and Weight Changes (g) During Dosing Period

Weight Parameter	Dosage (mg/kg/day)			
	0	1.0	2.5	10.0
	Males			
Mean Initial Bodyweight	11300	11317	11267	11433
Mean Wt. After 26 Weeks	12733	12900	13650	13233
Mean Gain (Weeks 1-26)	1433	1583	2383	1800
	Females			
Mean Initial Bodyweight	10583	10483	10467	10433
Mean Wt. After 26 Weeks	12983	13300	12567	12483
Mean Gain (Weeks 1-26)	2400	2817	2100	2050

Most dogs consumed all food offered. Small residues of remaining food was recorded. The following table summarizes food left.

Group Mean Total Quantities of Food Left During Pre-Dosing Period and During Study (Both Sexes Combined)

Group	Pre-Dose 4 Weeks	Weeks 1-26
Control	33	405
1.0 mg/kg/day	68	52
2.5 mg/kg/day	18	28
10.0 mg/kg/day	15	981*

*Statistically significant ($p < 0.05$) by Chi^2 test, based on a 2x2 contingency table. The proportion of control and 10.0 mg/kg/day dogs leaving food during the dosing period were compared.

Group Mean Serum Electrolyte and Urinary pH and Specific Gravity Values - Both Sexes Combined								
Parameter	Dosage mg/kg/day	Results Before Dosing Commenced	Results During Week					
			4	8	12	16	20	25
			of Dosing					
Sodium (Na) mEq/l	Control	145	146	145	148	148	148	148
	1.0	143*	146	147*	149	148	145*	149
	2.5	143**	143**	148**	147	149	145*	148
	10.0	144	144*	148**	148	147	145*	149
Potassium (K) mEq/l	Control	4.7	4.6	4.6	4.6	4.5	4.4	4.5
	1.0	4.6	4.6	4.7	4.7	4.6	4.5	4.5
	2.5	4.6	4.6	4.6	4.6	4.4	4.4	4.4
	10.0	4.8	4.7	4.8	4.7	4.5	4.5	4.4
Calcium (Ca) mEq/l	Control	5.5	5.4	5.4	5.4	5.4	5.7	5.4
	1.0	5.5	5.4	5.5	5.5	5.4	5.6	5.4
	2.5	5.6	5.4	5.5	5.5	5.4	5.6	5.4
	10.0	5.5	5.5	5.6	5.4	5.3	5.7	5.6*
Chloride (Cl) mEq/l	Control	109	111	113	113	112	116	112
	1.0	108	108*	114	114	113	115	111*
	2.5	108	110	113	113	111	112***	111
	10.0	108	110	112	112	110**	112***	111
Inorganic Phosphorus (P) mEq/l	Control	3.9	3.4	3.2	3.0	2.8	2.7	2.6
	1.0	3.8	3.5	3.3	3.1	3.0	2.7	2.6
	2.5	3.7	3.3	3.1	3.0	2.8	2.6	2.5
	10.0	3.8	3.4	3.3	3.0	2.8	2.7	2.6
Urinalysis Results								
Urinary pH	Control	6.0		6.3		6.7		6.3
	1.0	6.0		6.2		6.8		6.2
	2.5	6.0		6.3		6.8		6.3
	10.0	6.0		6.5		6.6		6.4
Urinary Volume ml	Control	159		125		147		126
	1.0	138		132		132		123
	2.5	125		121		130		125
	10.0	123		97		108*		129
Urinary Specific Gravity x 10^3	Control	1041		1045		1048		1046
	1.0	1041		1043		1049		1046
	2.5	1043		1046		1049		1048
	10.0	1042		1047		1048		1046

*p < 0.05
**p < 0.01
***p < 0.001

Intergroup Comparison of Microscopic Findings - Males

Observation	Treatment (mg/kg/day)			
	0	1.0	2.5	10.0
Kidney				
# Examined	6	6	6	6
Focal nephritis	2	0	0	0
Lymphocytic pyelitis renal scarring	0	0	1	0
Liver				
# Examined	6	6	6	6
Lipid in cytoplasm of bile duct epithelial cells	5	6	6	5
Testes				
# Examined	6	6	6	6
Occasional multinucleate cells in seminiferous tubules	1	2	2	4
Brain				
# Examined	6	6	6	6
Artefactual mucocyte vacuolation in White matter	1	4	2	
Pituitary				
# Examined	6	6	6	6
Anterior lobe cysts with basophilic colloid	4	4	5	5
Spinal Cord				
# Examined	6	6	6	6
Occasional swollen myelin sheath with myelophage	2			
Solitary swollen myelin sheath with central granular eosinophilic round body	1			
Agonal hemorrhage and artefactual vacuolation in white and/or grey matter		2		
Occasional myelin sheath vacuolation			2	
Occasional swollen myelin sheath with Gitter cells			2	

Intergroup Comparison of Microscopic Findings Females

Observation	Treatment (mg/kg/day)			
	0	1.0	2.5	10.5
Kidney				
# Examined	6	6	6	6
focal nephritis	1	1	0	0
Lymphocytic pyelitis renal scarring	0	1	0	2
Liver				
# Examined	6	6	6	6
Lipid in cytoplasm of bile duct epithelial cells	6	6	5	5
Cervix				
# Examined	6	6	6	6
Occasional neutrophilic leucocytes in epithelium	4	0	2	1
Brain				
# Examined	6	5	6	6
Artifactual vacuolation of hypothalamic neurophil	1			
Artefactual vacuolation and/or mucocytes in white matter		6		3
Agonal hemorrhage		1		
Stem: Occasional eosinophilic swellings (neuroaxonal dystrophy) in dorsal nuclei		1		
Artefactual vacuolation				1
Pituitary				
# Examined	6	6	6	6
Anterior lobe cysts contain basophilic colloid	6	5	6	3
Spinal Cord				
# Examined	6	6	6	6
Occasional myelin sheath swelling with myelophage	1			
Solitary glial focus in white matter of 1 section	1			
Occasional myelin sheath swelling with Gitter cells	2		3	2
Artefactual vacuolation in grey and/or white matter		3		
Artefactual vacuolation				1

Mouse Carcinogenicity Study Conducted with Cyhalothrin

Discussion of the Adequacy of the Dosing

At the previous RfD meeting on Cyhalothrin / Lambda-cyhalothrin, the Committee questioned whether or not the mouse carcinogenicity study was tested at sufficiently high dose levels. The Committee stated that "generally, the highest dose tested in the mouse carcinogenicity study appears to be approaching an adequate dose for carcinogenicity testing in males based upon decreased body weight gain. On the other hand, several questions were raised concerning the adequacy of doses tested....in females." The Registrant has submitted a response to the question concerning the dose levels tested and a 28-day range-finding study in the mouse. The full response is provided in Appendix IV and the Data Evaluation Record (DER) for the 28-day range-finding study is provided in Appendix V.

In their response, the Registrant stated that the 2-year mouse study was started in 1980, before records were kept on selection of dose levels. Therefore, they had to reconstruct the reasoning from the 28-day mouse study. They also stated that hypertrophy of the liver was not established to be of no toxicological significance at that time. In addition, the highest dose to be tested was set on the response of the most sensitive sex. "It was considered good practice not to have too wide a divergence in the dosing regimes used with the different species, since to do so was considered to impact adversely on extrapolations between species."

In the 28-day mouse study, cyhalothrin (technical, no purity available) was tested in an oral feeding study in CD-1 mice as a range-finding study for the carcinogenicity study. Twelve mice/sex/dose level were tested at 0, 5, 25, 100, 500 or 2000 ppm in the diet (0, 0.65, 3.30, 13.5, 64.2 or 309 mg/kg/day for males and 0, 0.80, 4.17, 15.2, 77.9 or 294 mg/kg/day for females).

At 2000 ppm, piloerection, abnormal gait (walking on toes), hunched posture, increase in respiration rate and emaciated appearance were observed. Six males and 3 females died during the study. Both males and females had a significant decrease in body weight gain over the treatment period when compared to controls (-1g versus 5 g in controls for males ($p < 0.001$) and 0g versus 3 g in controls for females ($p < 0.001$)). A decrease in food consumption was observed in both sexes during the first week (60.8% of controls for males and 62.5% of controls for females) and in females for the remainder of the study (82% of controls, $p < 0.05$). Males had a slightly lower mean total white blood cell count (68%). The differential white cell count revealed lower lymphocyte counts (58.7%) and higher neutrophil counts (62.5% above controls), $p < 0.01$ for all hematological values in males

Incidences of Mammary Adenocarcinomas in Female Mice Dosed With Cyhalothrin

0 ppm	20 ppm	100 ppm	500 ppm
1/52	0/52	7/52 (13.5%)	6/52 (11.5%)

In the paragraphs summarizing the data, the DER states that the incidences were statistically significant at 100 ppm ($p = 0.03$) and 500 ppm ($p = 0.04$). This was supported by a positive trend analysis ($p = 0.016$). However, there was a lack of a consistent dose-related response and the incidence at 100 ppm (13.5%) was only slightly higher than the laboratory's historical range (2-12%; average of 17 studies was 81/1156 or 7.0%). The incidence at 500 ppm (11.5%) was within the historical control range. In addition, as can be seen from the historical control data, the concurrent control was among the lowest of the historical control range, and the incidence of 0/53 tumors at the low dose of 20 ppm is lower than the lowest value observed in the historical control range. Neither the incidence at 100 ppm nor that at 500 ppm are statistically significant when compared to the historical control mean (Fishers Exact, $p = 0.16$, $p = 0.37$; statistics provided by Registrant).

The following table shows the historical control data on the incidence of mammary adenocarcinoma in studies performed on female CD-1 mice at the Huntingdon Research Centre for animal delivery dates between May 1978 and November 1980. Also included is the incidence of mammary adenocarcinoma in control female mice in this study (ICI 395).

at this dose level. Significantly higher APDM activity was observed in both sexes (61.9% above controls for males and 77.8% above controls for females). Slight increases in kidney weights (28.8% over controls for males, $p < 0.001$) and liver weights (17% over controls for males ($p < 0.05$) and 3.1 % over controls for females) were observed. In females, the differences were not statistically significant. Slightly lower heart weights were also observed in females (87.7% of controls, $p < 0.05$)). Minimal centrilobular hepatocyte enlargement was observed in 2/12 females.

At 500 ppm, piloerection was observed in several mice, several males had low white blood cell counts (not statistically significant) as well as marginally lower lymphocyte numbers (80%). Significantly higher APDM activity was observed in females (26.2% over controls). Slightly higher kidney weights were observed in males (13.5% over controls, $p < 0.01$)) and slightly lower heart weights were observed in females (93.0%, $p < 0.05$).

At 100 ppm, piloerection was also observed in several mice. One female had an emaciated appearance. Marginally lower lymphocyte numbers were noted for males (79% of controls). Significantly higher APDM activity was observed in females (24.8% over controls). Slightly higher kidney weights were observed in males (13.0% over controls, $p < 0.01$) and marginally lower heart weights were observed in females (87.7%, $p < 0.01$).

The NOEL is 500 ppm and the LEL is 2000 ppm based on mortality, clinical signs of toxicity, decreases in body weight gain and food consumption, changes in hematology and organ weights and minimal centrilobular hepatocyte enlargement. The minimal effects observed at 500 and 100 ppm are not considered to be toxicologically significant.

Thus, from this study, it is evident that 2000 ppm was above the MTD (mortality). The effects at 500 ppm were minimal. Therefore, it appears that the MTD for the mouse study is somewhere between 500 and 2000 ppm.

Discussion of the Incidences of Mammary Adenocarcinomas

At the previous RfD meeting, the Committee had questions concerning the significance of the mammary adenocarcinomas in female mice. The incidences were reported in the Data Evaluation Record (DER) as follows:

Historical Control Data on the Incidence of Mammary Adenocarcinoma				
Study	Date of Animal Receipt	Duration (Weeks)	Incidence*	
1	24/05/78	104	3/60	5.0%
2	31/05/78	104	7/60	11.7%
3	05/07/78	104	6/100	6.0%
4	30/08/78	108	3/51	5.9%
5	13/12/78	107	6/52	11.5%
6	14/02/79	104	1/55	1.8%
7	25/04/79	115	10/104	9.6%
8	04/07/79	121	9/104	8.7%
9	18/07/79	107	2/52	3.8%
10	29/08/79	104	3/52	5.8%
11	19/09/79	104	3/52	5.8%
12	05/03/80	108	3/52	5.8%
13	19/03/80	104	1/50	2.0%
14	19/03/80	111	5/104	4.8%
15	26/03/80	109	11/104	10.6%
16	18/06/80	108	5/52	9.6%
17	12/11/80	104	3/52	5.8%
Total ICI 135	12/3/80	104	81/1156 1/52	7.0% 1.9%

*Incidence is expressed as the number of mice with mammary adenocarcinoma over the number of control mice in the main group (excluding satellite group animals) and as a percentage.

The Registrant provided the following summary table on mouse adenocarcinomas in the mouse study:

Incidence at 2 Years of Mouse Adenocarcinoma

Dose (ppm)	0	20	100	500
Interim	0/12	0/11	0/11	0/10
Intercurrent	0/27	0/33	5/28 p=0.03	3/34 p=0.17
Terminal	1/25	0/20	2/25 p=0.50	3/20 p=0.22
Total *Corrected	1/64	0/64	7/64 p=0.03 *p=0.03	6.64 p=0.06 *p=0.04
Intercurrent Plus Terminal	1/52	0/53	7/53 p=0.03	6/54 p=0.06
Trend Test (Total)	*p=0.016			

P values quoted are for a Fishers Exact comparison with concurrent control values unless otherwise designated.

P values marked with an asterisk are from analyses corrected for inter-group differences in mortality and context of observation.

The Registrant also provided the following comments concerning the mammary adenocarcinomas in this study.

> "Zeneca believes that there is a compelling pathological case which demonstrates that the tumors are not treatment-related.
>
> The morphology of the tumors in the treated mice is identical to that of the mammary adenocarcinomas seen in the control mice in this and other studies. A difference in morphology might have been expected if the tumors were compound-related.
>
> All tumors occurred as single lesions in individual mice. There was no evidence of multiplicity of tumors with increasing dosage which might have been expected if they were compound-related.
>
> None of the tumors in treated animals showed evidence of metastases. The only tumor that had metastasized in this study was that in a control animal.
>
> There was no evidence of pre-neoplastic change in the mammary gland.

All tumors were self-evident (first seen as palpable masses) which permits good analysis of their onset. There was however no evidence of decreased latency or time of onset of the mammary tumors in comparison with controls.

The findings were restricted to one sex (female) and one species (mouse). Furthermore there is no published evidence that other pyrethroids induce mammary adenocarcinmoas in any species (Bradbury & Coats, 1989). **[Note: TB-1 checked the Division's Carcinogenicity Peer Review files on pyrethroids and verifies that there are no data at this time which indicate that this class of chemicals induces mammary tumors in mice.]**

In summary, Zeneca is led to conclude that there is no treatment-related effect on the incidence of mammary adenocarcinomas in this study based on the following:

(i) the absence of a dose-response relationship;

(ii) the low concurrent control incidence;

(iii) the incidence at a single intermediate dose is only marginally in excess of the historical control range and is not statistically significant when compared to historical controls;

(iv) the pathology of the lesion is entirely consistent with control (spontaneous) tumors. Zeneca concludes that the mouse mammary adenocarcinoma is not treatment-related."

Ref.: Bradbury S.P. and Coats J.R. (1989) Comparative Toxicology of the Pyrethroid Insecticides. Reviews of Environmental Contamination and Toxicology. Vol. 108 pp 133-177. Springer-Verlag Publishers, New York.

Appendix II: Discussion of Fluid Feces in Dogs With Pyrethroid Chemicals

Incidence of Fluid Feces

An increased incidence of fluid feces is not an uncommon clinical observation in response to dosing with pyrethroids. This change, which was also seen in the control beagles should be differentiated from true diarrhea because

a) the dogs often also pass normal feces on days when liquid feces are observed and

b) there is no increased frequency of defecation observed which would be expected with a true diarrhea.

The observation arises from one or two probable causes which could occur together or independently. These are

a) a decreased water absorption from the colon or

b) a decreased intestinal transit time.

The former is driven by active absorption of sodium with water following passively. Thus any local effect which might cause a reduction in the ability to concentrate ions against a concentration gradient could result in reduced absorption of water and hence increased fluidity of feces (Billich and Levitan 1969).

Gut motility (peristalsis) is under control of the autonomic nervous system. Modification of the conductivity of nerves involved in this process will alter gut motility and hence bowel transit time. For example some drugs used in the treatment of constipation will directly stimulate the nerve endings of the mucosa which causes the increase of muscle tone of the intestine (Hubacher and Doernberg 1964). This is a local effect which depends on continued presence of the stimulant.

The pyrethroid class of chemicals is known to change sodium permeability, at least in nerve membranes and possibly more generally (Bradbury et al 1983, Gray and Rickard 1982). Thus, it is probable that the ability of the pyrethroids to induce fluid feces under some circumstances can be explained by either a local effect on the nerve endings in the gut mucosa and/or by increasing sodium permeability in the colon. It should be noted that other pyrethroids, deltamethrin and cypermethrin, given by gavage over a 13 week period produced liquid feces inter alia. However, these changes were not seen in subsequent studies with dietary administration. (Buckwell and Butterworth, 1977; Kalinowski et al. 1982; FAO 1982).

This latter observation provides a probable explanation for the variable incidence seen. The compound was administered in a gelatine capsule shortly prior to the daily feed. It is predicted that dissolution of the capsule will not occur until after the dog has consumed its normal meal. Under these circumstances the test compound will be distributed through a relatively large volume of stomach contents. If, however, due to sporadic early dissolution or delayed feeding behavior, the capsule dissolves prior to the dog consuming its full daily feed, then the pyrethroid may well pass through the intestines as a bolus rather than as a more dilute form. In this latter case local pharmacological actions of the type described above are more probable because of the high localized concentration of the test compound within the gastrointestinal tract.

More detailed consideration of the findings in the lambda cyhalothrin study shows that as expected the major incidence of fluid feces was seen in the high dose group. The slightly increased incidence in the mid dose group was substantially attributable to one female(F33) with the other females and the males showing individual incidences similar to the control range. This pattern of response might be expected if feeding behavior was the major determinant, though it is notable that this dog also showed a higher incidence of fluid feces pre-study. There was no individual or group increase at the low dose.

In summary the phenomenon is believed to be due to a localized pharmacological reaction to a high concentration of a test compound and this would account for the apparent dose-response relationship observed in the study. As the observation is also made in control dogs it is not compound specific. It is readily reversible as demonstrated by the sporadic incidence in individual dogs and, as with all such pharmacological type reactions, it is without histological change, indicating that no permanent structural or functional change is induced. For these reasons the observation is considered to be of no toxicological significance.

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Appendix III: Discussion on the Incidence of Convulsions in 2 Dogs at 0.5 mg/kg/day in 1-Year Dog Study

Lambda-cyhalothrin
1-Year Dog Study
Guideline No. 83-1(b)

Incidence of Convulsions

Towards the end of the study, two males in the mid-dose group (0.5mg/kg/day) convulsed shortly after being transported. Dog M26 convulsed briefly in week 52 of the study while being carried to the room in which clinical examinations were performed by the Veterinary Officer. The next week, this dog again convulsed briefly while being carried to the clinical examination room, for its final examination prior to termination at the end of the study. Dog M27 convulsed twice, shortly after being put into a metabolism cage, in week 51, and again on the next day when it was removed from the metabolism cage. No other convulsions had been recorded in any dog in this dose group at any other time. Since on each of these occasions the dogs were being subjected to manipulative procedures, the Study Director concluded that these episodes were unrelated to compound, being induced by the stress and noise associated with handling. The following text gives further information to support this conclusion.

Convulsions are a rare event in dogs, but are known to occur in laboratory beagles. Examination of the clinical observation records at CTL have shown two instances of control dogs convulsing during manipulative procedures - one male dog convulsed during bone marrow sampling and a female dog (on another study) convulsed on being taken in to the clinical examination room, on week 53 of the study, just prior to termination. The situation with this latter dog is clearly directly comparable with that of M26 on the lambda-cyhalothrin study.

In addition, convulsions have been observed in a dog being dosed with a compound known NOT to be neurotoxic, as evidenced by unequivocal negative findings in acute and sub-chronic neurotoxicity studies in rats. In this case a single dog convulsed on six seperate occassions when taken to the clinical examination room for ophthalmoscopy. As with the other dogs, these convulsions came on later in the study (occurring between weeks 24 and 52). In this case, the relationship to the stress of being taken into the clinical examination room was amply demonstrated when the dog was examined at its pen-side, when it did not convulse.

Zeneca are confident that the conclusion of the Study Director was correct and that the two occasions of convulsions in two dogs in the lambda cyhalothrin study were unrelated to compound, being induced by the manipulative procedures, described above.

Appendix IV: Registrant Response to Dose Setting for Two Year Mouse Study

2. DOSE SETTING FOR TWO YEAR MOUSE STUDY

This study was started in 1980 before the universal practice of archiving a dose setting rationale with the raw data. Nevertheless with knowledge of the philosophy behind the then current practices and the results obtained in the 28 day study it is possible to reconstruct the logic used in the dose selection for the chronic study.

Background information

28 day studies were commonly used to set doses for life time studies in mice. These studies essentially posed the question, "Are the effects seen in the mouse qualitatively similarly to those in the rat?" If the answer was in the affirmative then additional information on progression of the toxic responses seen could be gained from the 90 day rat study, with the 28 day mouse study supplying essentially quantitative information to allow doses for the chronic study to be set.

Adaptive hypertrophy of the liver was not then unequivocally established as of no toxicological significance. It was therefore taken into account when setting doses although it was generally not regarded as the sole determinant of the highest dose.

It was usual practice to treat both sexes equally. Thus the highest dose would be set with reference to the sex showing the greater response. This remains laboratory practice unless the difference between the sexes is very large.

It was considered good practice not to have too wide a divergence in the dosing regimes used with the different species, since to do so was considered to impact adversely on extrapolations between species.

Results in the 28 Day Range-Finding Study

The mouse 28 day study showed significant mortality (50% in males and 25% in females) at 2000 ppm in diet and the animals gained no weight at all.

The next dose tested was 500 ppm in diet where the response was limited to pilo-erection in the males (also seen at lower doses) with a slightly reduced weight gain, compared to the lower dose groups, though not the controls (which showed an atypically small gain for male mice). In addition there was a small increase in kidney weight in males at this dose level together with some signs of increased amino-pyrene demethylase activity (APDM-a marker for some isoenzymes of cytochrome P_{450}) in the liver. By contrast females showed a clear and statistically significant increase in APDM activity at both 500 and 100 ppm.

These effects were qualitatively similar to those seen in the 90 day rat study, endorsing the approach that predictions for the progression of toxicity could be based on the rat data with those in the 28 day mouse study being used to determine dose levels. It should be noted that in the rat study body weight continued to diverge from controls after the initial 4 week period, albeit at a slow rate.

Conclusions from the 28 day Mouse study

It was evident that 2,000 ppm would be too high a dose for the chronic study, and that 500 ppm produced minimal responses in a 28 day period. In the absence of any additional information on the dose response relationships between these two doses and given their proximity it would have been considered imprudent to exceed the 500 ppm dose rate to any significant extent, especially as significant changes would be expected to develop further over the course of the chronic study. The expectation would have been that the sensitivity afforded by the increased numbers of animals used in a chronic study would be sufficient to establish 500 ppm as at or close to an MTD (as defined by OECD) probably in terms of body weight (a conclusion born out for males in the chronic study) and certainly in terms of increased liver (and possibly kidney) weight and increased APDM activity.

The low dose was selected at 20 ppm on the basis of increased APDM activity in females where it was considered that this would represent a no effect level. The mid dose (100 ppm) was selected as the geometric mean between 20 and 500 ppm.

It should be noted that these doses are exactly twice the inclusion rate of those used in the rat study. This means that the dose rates in (mg/kg/day) received by the mice were some five times greater than those for the rats.

Conclusions

Given the combination of factors detailed above, and the experience with contemporary studies, the information generated would have been considered sufficient for dose selection and we believe no other course of action would have been taken.

In the event, it is clear that an MTD, as defined in 1980, was achieved for male mice and that female mice were close to this level (by less than a four-fold factor).

5-26-94

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Secondary Reviewer: Roger L. Gardner, Section Head Roger Gardner 5/26/94
Section I, Tox. Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 4-Week Dose Range Finding Study in Mice

SHAUGHNESSY NO./TOX. CHEM. NO.: 725C, 271F / 128897

TEST MATERIAL: Cyhalothrin

SYNONYMS: PP563

REPORT NUMBER: CTL/C/1039

SPONSOR: Imperial Chemical Industries, Cheshire, England

TESTING FACILITY: Huntingdon Research Center, Huntingdon, Cambridgeshire, England

TITLE OF REPORT: Cyhalothrin: 4-Week Dose Range Finding Study in Mice

AUTHOR(S): J. C. Colley, S. Dawe, R. Heywood, W. Dawn, R. Woodhouse, C. Gopinath, A. Zubaidy, D. Prentice

REPORT ISSUED: 2/27/81

CONCLUSION: Cyhalothrin (technical, no purity available) was tested in a 4-week oral feeding study in CD-1 mice as a range-finding study for the carcinogenicity study. Twelve mice/sex/dose level were tested at 0, 5, 25, 100, 500 or 2000 ppm in the diet (0, 0.65, 3.30, 13.5, 64.2 or 309 mg/kg/day for males and 0, 0.80, 4.17, 15.2, 77.9 or 294 mg/kg/day for females).

At 2000 ppm, piloerection, abnormal gait (walking on toes), hunched posture, increase in respiration rate and emaciated appearance were observed. Six males and 3 females died during the study. Both males and females had a significant decrease in body weight gain over the treatment period when compared to controls (-1g versus 5 g in controls for males ($p < 0.001$) and 0g versus 3 g in controls for females ($p < 0.001$)). A decrease in food consumption was observed in both sexes during the first week (60.8% of controls for males and 62.5% of controls for females) and in females for the remainder of the study (82% of controls, $p < 0.05$). Males had a slightly lower mean total white blood cell count (68%). The differential white cell count revealed lower lymphocyte counts (58.7%) and higher neutrophil counts (62.5% above controls), $p < 0.01$ for all hematological values in males at this dose level. Significantly higher APDM activity was

observed in both sexes (61.9% above controls for males and 77.8% above controls for females). Slight increases in kidney weights (28.8% over controls for males, $p < 0.001$) and liver weights (17% over controls for males ($p < 0.05$) and 3.1 % over controls for females) were observed. In females, the differences were not statistically significant. Slightly lower heart weights were also observed in females (87.7% of controls, $p < 0.05$)). Minimal centrilobular hepatocyte enlargement was observed in 2/12 females.

At 500 ppm, piloerection was observed in several mice, several males had low white blood cell counts (not statistically significant) as well as marginally lower lymphocyte numbers (80%). Significantly higher APDM activity was observed in females (26.2% over controls). Slightly higher kidney weights were observed in males (13.5% over controls, $p < 0.01$)) and slightly lower heart weights were observed in females (93.0%, $p < 0.05$).

At 100 ppm, piloerection was also observed in several mice. One female had an emaciated appearance. Marginally lower lymphocyte numbers were noted for males (79% of controls). Significantly higher APDM activity was observed in females (24.8% over controls). Slightly higher kidney weights were observed in males (13.0% over controls, $p < 0.01$) and marginally lower heart weights were observed in females (87.7%, $p < 0.01$).

The NOEL is 500 ppm and the LEL is 2000 ppm based on mortality, clinical signs of toxicity, decreases in body weight gain and food consumption, changes in hematology and organ weights and minimal centrilobular hepatocyte enlargement. The minimal effects observed at 500 and 100 ppm are not considered to be toxicologically significant.

This study is not a guideline requirement and thus does not satisfy any guideline requirements.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: (RS)-α-cyano-3-phenoxybenzyl (1RS)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropanecarboxylate

Description: Brown viscous liquid
Batch #: Y00102/010/001
Purity: Not specified
Source: Central Toxicology Laboratories, ICI Ltd., England

2. Test Animals:

Species and Strain (sexes): Male and female CD-1 mice
Age: 24 ± 1 day
Weight(s): 22-23 g (mean) - ♂; 20-21 g (mean) - ♀
Source(s): Charles River, Manston, Kent, England

3. Procedure:

a. Dietary Preparation: A premix was prepared by mixing the test material in corn oil and then diluting it with test diet to the specified amounts.

Frequency of preparation: The premix was diluted to the appropriate concentration weekly.

Storage conditions: Not stated.

Stability Analyses: An analysis at the 5 and 2000 ppm dietary levels was conducted using the diet prepared at week 1. The samples were stored in darkness at ambient temperatures in the animal rooms for up to 18 days. Duplicate sub-samples were prepared after 9 and 18 days storage and analyzed.

Homogeneity Analyses: An analysis at the 5 and 2000 ppm dietary levels was conducted using the diet prepared at week 1. Duplicate samples were taken from the first kilogram discharged, from the approximate center of the discharge and from the final kilogram discharged.

Concentration Analyses: Concentration analyses were conducted in weeks 1 and 4.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of previous studies.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 4 Weeks male	female
Contr.	0	12	12
1	5	12	12
2	25	12	12
3	100	12	12
4	500	12	12
5	2000	12	12

d. Clinical Observations and Mortality: All animals were checked twice daily for clinical signs of toxicity and mortality.

e. Body Weight Determinations: Weekly.

f. Food and/or Water Consumption: Weekly.

g. Clinical Pathology:

1) Hematology:

Collection times for blood (including # of animals): During week 4, blood samples were taken from all animals under light anesthesia from the orbital sinus.

The following CHECKED (X) parameters were examined:

X		X	
	Hematocrit (HCT)	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (Hb)	x	Mean corpuscular HGB conc. (MCHC)
x	Leukocyte count (WBC)	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)	x	Reticulocytes
x	Platelet count	x	Packed cell volume (PCV)
	Total plasma protein (TP)		
x	Leukocyte differential count		

2) Urinalysis:

Collection times for urine (including # of animals): During week 4, overnight pooled urine samples were collected from 4 mice of each sex/group. Food was removed. The urine samples were stored for proof of absorption studies.

3) Aminopyrine Demethylase Activity

Aminopyrene demethylation (APDM) assays were conducted on 6 animals/sex/dose using liver homogenate as the enzyme source. The results were expressed as nmoles formaldehyde produced/hour/gram liver.

h. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: All animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals.

i. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: All animals.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: All controls and high dose animals. Livers from all groups were examined.

Samples of liver approximately 1 mm^3 were obtained from 6 male and 6 female mice/group and fixed in paraformaldehyde-glutaraldehyde fixative and post-fixed in 1% osmium tetroxide. The tissues were dehydrated and embedded in epoxy resin. One μm survey sections were cut and stained with toluidine blue for examination with the light microscope. Silver/gold ultra thin sections of selected areas were cut, mounted on copper grids and stained with uranyl acetate and lead citrate. The ultra thin sections were examined with a Philips EM 300 at an accelerating voltage of 80kv. A qualitative assessment of the smooth endoplasmic reticulum activity was made.

Where practicable, a section of kidney was stained with Periodic Acid Schiff (for basement membranes) and frozen sections of liver and kidney, fixed in buffered formalin, were cut on a cryostat at 12 μm and stained for fat with Oil Red O. In addition, sections of sciatic nerve and posterior nerve from 5/sex/group from the control and high dose groups were stained with Glees-Marsland silver stain and Luxol Fast Blue.

CHECKED (X) tissues were preserved for histopathological examination in buffered 10% formalin and (XX) tissues were weighed upon removal from the animal. These were embedded in paraffin wax and sections were cut at 5μ, stained with H & E and examined.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
x	Tongue	x	Aorta	xx	Brain
x	Salivary glands	xx	Heart	x	Sciatic nerve
x	Esophagus	x	Bone marrow		Spinal cord
				x	Posterior tibial nerve
x	Stomach	x	Lymph nodes	x	Pituitary
x	Duodenum	x	Spleen	x	Eyes (optic n.)
x	Jejunum	x	Thymus		Glandular
x	Ileum		Urogenital	xx	Adrenals
x	Cecum	xx	Kidneys		Lacrimal gland
x	Colon	x	Urinary bladder	x	Mammary gland
	Rectum	xx	Testes	x	Parathyroids
xx	Liver		Epididymides	x	Thyroids
x	Gall bladder	x	Prostate		Other
x	Pancreas	x	Seminal vesicle	x	Bone
	Respiratory	xx	Ovaries	x	Skeletal muscle
x	Trachea	x	Uterus + Cervix	x	Skin
xx	Lung				All gross lesions and masses

j. Statistical Analyses: An analysis of variance followed by Student's 't' test was performed to assess the significance of intergroup differences in bodyweight, food intake and APDM. Analysis of hematological investigations were performed using analysis of variance followed by Williams' test. Analysis of organ weights was performed using analysis of covariance.

B. RESULTS:

1. Dietary Preparation: An analysis of the dosing concentrations from week 1 revealed a range of -15.0% to +3.6% of the nominal concentrations. At week 4, the range was -1.9% to +5.6%. The -15.0% was for the 500 ppm dose level. All the other dose levels were within

the 5.6% range. The homogeneity results indicated a range of 4.98 to 5.24 ppm for a dose level of 5 ppm and 1920 to 1990 ppm for a dose level of 2000 ppm. The stability study indicated that the test material was stable for a period of 18 days. After 18 days, the concentration at the 5 ppm dose level was 5.18 ppm and the concentration at the 2000 ppm dose level was 1970 ppm.

2. Clinical Observations and Mortality: At 2000 ppm, the following clinical signs were observed: piloerection, abnormal gait (walking on toes), hunched posture, increase in respiration rate and emaciated appearance. Piloerection was also observed in several mice at 25, 100 or 500 ppm, for 1 male at 5 ppm and for 1 control male. One female at 100 ppm had an emaciated appearance. There was no summary table in the report for these observations.

   Six males and 3 females in the 2000 ppm group died during the study. Autopsy examinations revealed congested lungs and autolytic changes in the abdominal viscera for 1 mouse, small thymus and spleen for a second mouse and small spleen in a third mouse. Microscopic examination of the small spleens revealed atrophy of the red pulp, which according to the authors was of unknown toxicological significance.

3. Body Weight Determinations: At 2000 ppm, both males and females had a significant decrease in body weight gain over the treatment period. Males lost weight. No effect was observed at any of the lower dose levels. The following table summarizes the results.

Group Mean Bodyweights (g)

	Dose Levels (ppm)					
Week	0	5	25	100	500	2000
			Males			
-1	23	22	22	23	23	23
0	27	27	26	26	26	26
1	29	31	30	31	28	23
2	31	33	31	32	30	25
3	32	34	32	33	32	24
4	32	35	34	35	33	25
Weight Gain 0-4	5	8**	8**	9**	7	-1***

Group Mean Bodyweights (g)

Week	Dose Levels (ppm) 0	5	25	100	500	2000
			Females			
-1	21	21	21	20	21	20
0	23	23	23	22	23	22
1	24	25	24	24	24	21
2	25	26	25	25	25	21
3	24	25	25	26	25	21
4	26	27	26	27	26	22
Weight Gain 0-4	3	4	3	5	3	0***

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

4. Food Consumption: At 2000 ppm, a decrease in food consumption was observed in both sexes during the first week and in females for the remainder of the study. The group mean achieved compound intakes were as follows: 0, 0.65, 3.30, 13.5, 64.2 or 309 mg/kg/day for males and 0, 0.80, 4.17, 15.2, 77.9 or 294 mg/kg/day for females. The following table summarizes the results.

Group Mean Food Consumption (g/mouse/week)

Week	Dose Levels (ppm) 0	5	25	100	500	2000
			Males			
1[a]	23	27	25	29	21	14
2	27	27	27	28	28	26
3	27	31	30	31	29	31
4	27	32	31	31	29	32
Total	104	117	113	119	107	103
% of Control	-	113	109	114	103	99

Group Mean Food Consumption (g/mouse/week)

Week	Dose Levels (ppm) 0	5	25	100	500	2000
			Females			
1[a]	24	24	27	22	24	15
2	26	27	28	26	26	20
3	25	28	27	28	25	24
4	29	32	30	29	30	26
Total	104	111	112	105	105	85
% of Control	-	107	108	101	101	82*

[a]Food consumption for a 6-day period.
*p < 0.05 when compared to control value.

5. Hematology: At 2000 ppm, males had a slightly lower mean total white blood cell count. This was particularly true for 3 males. The differential white cell count revealed lower lymphocyte counts and higher neutrophil counts in males at this dose level. At 500 ppm, 2 males had low white blood cell counts that were similar to the high dose males. Marginally lower lymphocyte numbers were noted for males at this dose level and at 100 ppm. The report also stated that marginally lower PCV and Hb values were observed in high dose males, however, these were largely due to low values recorded for 1 mouse. No significant changes were observed in females. The following table summarizes these data.

Group Mean Values for Total White Blood Cell, Lymphocyte and Neutrophil Counts in Males

Dose Levels (ppm)	Total WBC	Lymphocyte	Neutrophil
0	8.5 ± 2.30	7.5 ± 2.16	0.8 ± 0.36
5	7.7 ± 1.41	6.7 ± 1.21	0.9 ± 0.26
25	7.1 ± 1.87	6.2 ± 1.87	0.9 ± 0.37
100	6.7 ± 2.34	5.9 ± 1.92*	0.8 ± 0.42
500	7.0 ± 1.68	6.0 ± 1.46*	0.9 ± 0.30
2000	5.8 ± 2.28**	4.4 ± 1.92**	1.3 ± 0.62**

* p < 0.05
** p < 0.01

6. Aminopyrine Demethylase (APDM) Activity: Significantly higher APDM activity was observed in high dose males and in 100, 500 or 2000 ppm females. The following table summarizes the data.

Mean Aminopyrine Demethylase Activity (μ mole/hr/g liver)

Dose Group (ppm)	Males	Females
0	48.0	50.4
5	40.6	54.3
25	47.4	58.3
100	44.3	62.9*
500	53.3	63.6*
2000	77.7***	89.6***

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

7. Gross Pathology: No treatment-related differences were observed between the treated groups and the control groups.

8. Organ Weights: At 2000 ppm, slight increases in kidney and liver weights were observed in both sexes. In females, the differences were not statistically significant. Slightly lower heart weights were also observed in females. At 500 ppm, slightly higher kidney weights were observed in males and slightly lower heart weights were observed in females. At 100 ppm, slightly higher kidney weights were observed in males and marginally lower heart weights were observed in females. At 25 ppm, slightly higher liver weights were observed in males. This was not considered to be biologically signficant because it was not dose-related. The following table summarizes the results.

Group Mean Organ Weights (g)

Dose Level (ppm)	Bodyweight	Heart	Liver	Kidneys
		Males		
0	32	0.153 (0.150)	1.783 (1.733)	0.525 (0.515)
5	35	0.153 (0.163)	1.656 (1.858)	0.520 (0.559)
25	33	0.167 (0.169)	2.028[b] (2.067)	0.572 (0.579)
100	33	0.172[a] (0.172)	1.881 (1.873)	0.593[b,e] (0.591)
500	33	0.159 (0.162)	1.949 (2.003)	0.596[b,e] (0.607)
2000	26	0.172 (0.149)	2.088[d] (1.613)	0.676[c,e] (0.584)

Group Mean Organ Weights (g)

Dose Level (ppm)	Bodyweight	Heart	Liver	Kidneys
		Females		
0	26	0.138 (0.139)	1.576 (1.607)	0.418 (0.424)
5	26	0.129 (0.132)	1.499 (1.549)	0.403 (0.413)
25	27	0.127 (0.131)	1.492 (1.572)	0.393 (0.409)
100	26	0.121[b,d] (0.122)	1.439[a] (1.470)	0.385 (0.391)
500	26	0.128[d] (0.130)	1.558 (1.589)	0.405 (0.411)
2000	22	0.121[a,d] 0.108	1.625 (1.328)	0.433 (0.372)

Where values adjusted are for final bodyweight as covariate absolute values are given in parenthesis.
[a]p < 0.05 't' test
[b]p < 0.01 't' test
[c]p < 0.001 't' test
[d]p < 0.05 Williams' test
[e]p < 0.01 Williams' test

9. Histopathology: No summary tables were provided. However, the findings were written in script. Minimal centrilobular hepatocyte enlargement was observed in 2/12 females in the 2000 ppm dose group. No other significant differences between the treated and control groups were observed that may account for the organ weight changes. None of the findings were considered to be of toxicological significance.

In the liver, the following observations were noted by the authors of the report:

"a base-line change characterized by minimal fine cytoplasmic vacuolation of hepatocytes (mainly periportal in females and centrilobular in males) with or without foci of parenchymal and/or periportal mononuclear aggregates, also occasional vacuolated and distended hepatocytes or sinusoidal engorgement in a large proportion of mice from control and top dose groups;

foci of hepatocellular degeneration/necrosis with associated inflammatory cells in one or more lobes, haphazard distribution: 1 male, 1 female control; 1 male (100 ppm); 2 males (2000 ppm).

with Oil Red O trace fat droplets noted in occasional mice from control and treated groups;

minimal fat deposits in centrilobular areas: 2 males control; 4 males (5 ppm); 1 female (25 ppm); and in periportal areas: 4 female control; 1 female (100 ppm).

a distended portal area with minimal biliary hyperplasia and fibrosis: 1 male and female (100 ppm)."

In the kidneys, the following observations were noted:

"Minimal peripelvic mononuclear aggregates in a few mice from control and high dose groups.

a few basophilic cortical tubules in one male and one female at 2000 ppm.

a few foci of mineralization in the papilla of one high dose male mouse.

congested intertubular blood vessels and glomerular capillaries, possibly hypervolemic in 1 high dose male mouse."

10. Quality Assurance Measures: The study was conducted prior to the GLP requirements.

C. DISCUSSION: This study was conducted in order to determine a suitable high dose level for the carcinogenicity study in the mouse. It is not a guideline requirement and thus does not satisfy any requirements.

Caswell No. 725C, 271F
Chemical Name Cyhalothrin
Shaughnessy No.: 128897

File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
4-Week Oral Feeding Study - Mice/ Huntingdon Research Center/CTL/C/1039; 2/27/81	Cyhalothrin (Tech). Y00102/010/001	Not available at this time	CD-1 mice: 12/sex/dose at 0, 5, 25, 100, 500, 2000 ppm (0, 0.65, 3.30, 13.5, 64.2, 309 mg/kg/day (♂); 0, 0.80, 4.17, 15.2, 77.9, 294 mg/kg/day (♀)). 2000 ppm: piloerection, abnormal gait (walking on toes), hunched posture, ↑ respiration rate & emaciated appearance. Six ♂ & 3 ♀ died during study. ♂+♀: ↓ bw gain. ↓ food consumption (♂+♀) during first week & in ♀ for remainder of study. ♂: slightly lower total WBC count. Differential white cell count: lower lymphocyte counts & higher neutrophil counts. ↑ APDM activity (♂+♀). Sl. ↑ kidney & liver wts (♂). Slightly lower heart weights (♀). Minimal centrilobular hepatocyte enlargement observed in 2/12 females. Minimal effects observed at 500 & 100 ppm not considered to be toxicologically significant. NOEL: 500 ppm LEL: 2000 ppm.	N/A	Supplementary

46